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The optimal protocol for measuring an albuminuria class transition in clinical trials in diabetic kidney disease

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Abstract

Introduction: Albuminuria class transition (normo- to micro- to macroalbuminuria) is used as an intermediate ~~surrogate~~ endpoint to assess renoprotective drug efficacy. Current definitions of such class transition vary between trials in: number of urine collections, requirement of a confirmation visit and if so at what time, and the requirement of a minimal percentage albuminuria from baseline. We evaluated these different approaches to obtain the most optimal protocol.

Methods: Four clinical trials testing the effect of renin-angiotensin-aldosterone-system intervention on albuminuria class transition in diabetic patients were included: BENEDICT, DIRECT, ALTITUDE and IRMA-2. Each trial used a different class transition definition based on: 1) either 1, 2, or 3 consecutively collected urine samples at each study visit, 2) different time intervals between study visits, 3) requirement of an additional visit to confirm the class transition, and 4) requirement of a percentage increase in albuminuria from baseline in addition to the class transition. We tested the effect of these different definitions on drug efficacy outcomes by Cox regression analysis.

Results: Neither increasing the number of urines collected at a single study visit, nor the time interval between study visits, nor the requirement of a subsequent confirmation visit, nor the addition of a minimal percentage albuminuria change from baseline altered the average drug effect. However, the standard error of the treatment effect increased (decreased precision) with stricter endpoint definitions resulting in a loss of statistical significance.

Conclusion: The optimal albuminuria transition endpoint for use in drug intervention trials can use a single urine collection for albuminuria assessment per study visit. A~~, without~~ confirmation of the end point, ~~and~~ or ~~with no~~a requirement of a minimal percentage change in albuminuria from baseline is not necessary.

Introduction

Transition in albuminuria class (normo- to micro- or micro- to macroalbuminuria) is a hallmark of progression of diabetic kidney disease.[1,2] This hallmark has been used as an endpoint in clinical trials to assess renoprotective efficacy of clinical interventions. Since there is no standardized protocol how to measure an albuminuria class transition, different definitions have been used in past trials[3,4,5,6,7,8] , varying in the way urine was collected (first morning void vs. spot urine),[3,8] how albuminuria was expressed (urinary albumin excretion or albumin:creatinine ratio),[3,6,8] the frequency and interval of urine collections to define a class transition,[4,3,6] as well as the requirement for a confirmatory visit.[3,4] Confirmation at a subsequent visit may be important, since albuminuria is known to show a large day-to-day variability.[9,10] To ensure that a class change is not due to chance, the end point definition is often complemented with a requirement that albuminuria increase should be greater than a predefined percentage of baseline.[3,4]

Harmonization of the endpoint criteria for the optimal definition of class transition in albuminuria is important for the design of future clinical trials. Defining a transition by a single measurement and without a predefined percentage change may increase the number of endpoints and thereby the statistical power to assess drug effects. However, a predefined percentage change and a subsequent confirmatory visit may decrease the number of endpoints but may increase the precision of the transition and thereby increase statistical power to assess drug effects.

The aim of this study was therefore to weigh the importance of all these opposing effects in a post-hoc analysis of completed clinical trials, which tested the effects of RAAS-inhibition on albuminuria class transitions. The objective was to determine the optimal definition for a transition in albuminuria class regarding the frequency of urine collections, the interval between study visits for urine collections, the requirement of confirmatory visits, and the cut-off threshold for the percentage change from baseline.

Results

Baseline characteristics of included trials are published elsewhere and are summarized in Table 1.[3,11,12,13]

Number of endpoints

Increasing the number of urine collections per visit reduced the number of endpoints (Table 2). The number of endpoints further decreased in each trial when the transition endpoint required

a confirmation at a subsequent visit (Table 2). Adding increasing percentages of albuminuria change to the endpoint definition only marginally reduced the number of endpoints in each trial (Table 2).

Number of urine collections at a single visit and time interval between visits

Increasing the number of urine collections at a single visit only had minimal effect on the average treatment effect and 95% confidence interval in the BENEDICT, DIRECT and ALTITUDE trials (Figure 1). For example, in the BENEDICT trial, the hazard ratio was 0.60 (95%CI 0.40 to 0.89) when the transition endpoints were calculated from the geometric mean of three consecutive urine samples at each visit and 0.66 (95%CI 0.46 to 0.99) based on single urine samples. Increasing the time interval between study visits for albuminuria assessment had no effect on the magnitude of the average treatment effect while the precision of the drug effect estimate decreased somewhat as indicated by the wider confidence intervals (Supplement Figure 1).

Requirement for confirmation visits and time interval of the confirmation visit

Addition of a confirmation visit to firmly establish the class transition only minimally affected the average treatment effects of trandolapril, candesartan, irbesartan or aliskiren (Figure 2). However, the precision of the drug effect estimate markedly decreased as reflected by the wider 95% confidence interval in all trials. For example, in the BENEDICT study, the hazard ratio was 0.66 (95%CI 0.46 to 0.99) when the transition endpoints were not confirmed and 0.60 (95%CI 0.21 to 1.70) with confirmation. Despite the different time intervals of the confirmatory visit, a decrease in precision occurred in all trials suggesting that the timing of the confirmatory visit had no impact on the result.

Percentage increase in albuminuria

Enhancing the level of minimal percentage change in albuminuria from baseline from 10% to ~~40~~100% had minimal impact on the average and standard error of the treatment effects of trandolapril, candesartan, irbesartan or aliskiren (Figure 3). For example in the BENEDICT study, the hazard ratio was 0.66 (95%CI 0.46 to 0.94) with 10% increase in albuminuria in addition to the class transition, ~~and~~ 0.~~68~~ 65 (95%CI 0.~~46~~ 44 to 0.94) with 5~~4~~0% increase in albuminuria, and 0.54 (95%CI 0.36 to 0.81) with 100% increase in albuminuria. Hazard ratio's were somewhat higher (smaller treatment effect) in most trials if the endpoint was based on a 50% or 100% increase without class transition (Supplement Table 2).

Association between albuminuria class transition endpoints and clinical outcomes

The ALTITUDE database was used to determine the association between a class transition in albuminuria, an intermediate endpoint, with subsequent clinical renal and cardiovascular outcomes. A transition in albuminuria stage from normo- to microalbuminuria or micro- to macroalbuminuria based on a single urine sample at a single visit was independently associated with a higher risk of the renal (HR 1.92 (95% CI 1.09 – 3.40; p=0·025) and cardiovascular endpoint (HR 1.45 (95% CI 1.15 – 1.83; p=0·002; Table 3). Hazard ratio's only marginally changed when the class transition was based on the average of multiple urine samples at a single visit, or when a confirmation visit or a percentage albuminuria increase to the endpoint definitions were added to define the class transition (Table 3).

Discussion

Transition of albuminuria class may become a more and more important (surrogate) end point to evaluate renoprotective drug effects. Its current use shows a wide variety of definitions of such an albuminuria class transition. The variations concerned the number of albuminuria collections per visit, the interval of study visits for albuminuria collection, the addition of a class-change-conformation visit, and the addition of a minimal percentage albuminuria change with the class transition. We established in a post hoc analysis of four randomized controlled clinical trials that none of these variations had a significant impact on most of the outcome parameters in the different studies. These data suggest that single urine collections at a study visit are sufficient to define a transition in albuminuria as an endpoint in clinical trials.

Because of the within individual day-to-day fluctuations in albuminuria prior clinical trials have used the average albuminuria from three consecutive instead of one urine collection at each study visit to determine a transition in albuminuria stage. In our study we found that decreasing the number of urine samples at a single visit led to a small increase in the number of endpoints, but the average drug effect and the precision of the drug effect did not change. It is likely that when using single urine samples a potential increase in the precision of the drug effect estimate (as a result of the higher number of endpoints) is balanced by introducing random noise and false positive endpoints so that the average drug effect and precision does not change. ~~An increase in the number of endpoints would be expected to result in an increase in precision of the drug effect estimate (decreased standard error) and increase in statistical significance. Yet, this did not occur possibly because using single compared to triple urine samples to define the endpoint may have introduced random noise and false positive endpoints.~~

~~These two effects may have balanced each other so that the average and confidence intervals of the drug effect estimate did not change. This finding is in line with our previous study demonstrating that increasing the number of urine samples at a single visit had a modest effect on the precision of the albuminuria lowering drug effect estimate~~

~~The results suggest that a time interval of 6 months between study visits resulted in treatment effects of larger statistical significance compared to a 12 month time interval between study visits.~~ ~~Unfortunately, urine collections did not occur more frequently than every 6 months in all trials. We were therefore unable to determine whether shorter time-intervals between visits would lead to more significant treatment effects.~~ ~~This needs to be addressed in future studies.~~

Another approach ~~that has been used in clinical trials~~ to ~~attenuate~~ minimize the impact of the ~~within individual~~ day-to-day fluctuations in albuminuria on the transition endpoint has been the requirement of a confirmation of the albuminuria transition. The addition of a confirmatory visit is expected to decrease the random noise in the treatment effect due to albuminuria fluctuations and false positive transitions. However, our analysis showed that adding a confirmatory visit to the endpoint definition led to a marked decrease in endpoints and decrease in precision of the drug effect estimates. The marked decrease in endpoints apparently overruled the potential gain in statistical power due to the removal of false-positive transitions. ~~The timing of the confirmation visit for the albuminuria transition differed in each trial but the results were consistent regardless at what time the confirmation visit was conducted.~~

Previous trials have added a 30% increase in albuminuria when the transition occurs as a third strategy to reduce the influence of day-to-day albuminuria fluctuations and endpoint occurrence due to chance, particularly for patients whose baseline albuminuria levels are close to the transition threshold. The first trial that added a 30% albuminuria increase ~~in albuminuria when the~~ to the transition endpoint ~~occurs~~ was a trial on the effect of captopril in patients with type 1 diabetes.[14] Although it seems logical to add a~~n~~ percentage increase in albuminuria to the endpoint definition ~~to avoid that people close to the border of a class, can have class change with very small albuminuria changes,~~ our results indicate that it had no effect on the number of endpoints, the average drug effect, nor on the precision of the drug effect.

The question is whether a transition in albuminuria class, a surrogate, represents a hard outcome, and if so, whether a single-urine-determined-transition is as good as two or three-consecutive-urine determined transitions for hard outcome prediction. Indeed in a post-hoc analysis of the ALTITUDE trial we found that a transition in albuminuria predicts renal and

cardiovascular outcomes ~~(Supplement Table 1)~~. In addition, the ~~predictive power~~association was independent of all different definitions that we used for the albuminuria class transition ~~(Supplement Table 1)~~. These data support using single urine samples at a single visit to define a transition endpoint.

Standardization of clinical trial endpoint definitions is important for drug regulators, physicians, and patients to assess and compare clinical trial results and to interpret drug efficacy. Surprisingly, all past clinical trials have used different definitions for a class transition in albuminuria endpoint. ~~The trials differed in the number of urine collections at each visit to determine urinary albumin excretion, the method of urine collection (timed overnight collections, first morning void collections), correction of urinary albumin concentration by urinary creatinine concentration, and requirement of a confirmation of the albuminuria transition.~~ Due to the different definitions we unified the endpoint definitions across all included trials in order to compare different trial results. Since we standardized the endpoint definitions for the included trials, our results may differ from the published results in the original publications.

These results may at first sight contrast our previous findings in which we concluded that increasing the frequency of urine collections during follow-up of a clinical trial increases the precision and statistical power to detect an albuminuria lowering drug effect.[15] However, there are important differences between these studies. Firstly, the endpoints between the studies were different. In the present study the drug effect on a dichotomous outcome was determined. In trials with dichotomous outcomes the statistical power is determined by the proportion of patients who reach the outcome. In our prior study we evaluated the drug effect on the continuous outcome of percentage albuminuria change. In these trials the power is determined by the magnitude of change and standard deviation of the change. ~~Second, the number of confirmation visits was different. In the current study we only used a single confirmation study whereas in our prior study we used multiple follow-up visits with urine collections. Whether confirmation of the transition endpoint at multiple visits would alter the results requires a study with multiple follow-up visits within a few months and this remains to be determined.~~ In addition, the populations were different. In the current study we included patients with normo- and microalbuminuria as these individuals may progress in albuminuria stage. In our previous study patients with macroalbuminuria were included. Noteworthy, using single urine samples for albuminuria measurements without confirmation may increase the sensitivity to detect a transition but decrease specificity resulting in false positive and/or false negative results. In the context of a randomized controlled clinical trial this may increase data variability without

affecting the overall result of the trial. For the individual patient treated in clinical practice the increase in false positive and/or false negative results may hamper diagnostic and prognostic performance and may lead to erroneous conclusions whether the individual is at increased renal or cardiovascular risk.

The limitations of our study are that we were only able to include clinical trials that assessed the effect of RAAS-intervention in patients with type 1 or type 2 diabetes. We do not know whether the results will be similar with other drugs or in other populations. To calculate a percentage increase in albuminuria we used the albuminuria values collected at a single baseline visit. Albuminuria was not assessed at multiple visits before randomization. We therefore do not know whether multiple baseline visits improved the precision in the percentage increase in albuminuria and the potential impact on the results. Thirdly, urine collections did not occur more frequently than every 6 months in all trials. We were therefore unable to determine whether shorter time-intervals between visits would lead to more significant treatment effects. Finally, these results were derived post-hoc. We encourage clinical trialists to prospectively validate these findings.

In conclusion, neither increasing the number of urines collected at a single study visit, nor the inclusion of a confirmation visit, nor the time to the confirmation visit, nor the addition of a minimal required percentage albuminuria change altered the average drug effect. Therefore, we conclude that future clinical trials in diabetic nephropathy using albuminuria transitions as endpoint should consider single urine collections per study visit spaced no more than 6 months apart for albuminuria assessment.

Concise methods

Patients and clinical trials

Data from four randomized controlled clinical trials in patients with type 1 or type 2 diabetes were analyzed: BENEDICT (Bergamo Nephrologic Diabetes Complications Trial), DIRECT (The Diabetic Retinopathy Candesartan Trials), ALTITUDE (Aliskiren Trial in Type 2 Diabetes Using Cardio-renal Endpoints), and IRMA-2 (Irbesartan in patients with type 2 diabetes and Microalbuminuria) trials were included in this study. All trials assessed the effect of intervention in the renin-angiotensin-aldosterone-system on progression of albuminuria. The study designs and results of each trial are reported elsewhere and here briefly described [3,6,11,12,16].

In the BENEDICT trial 1209 patients with type 2 diabetes, normoalbuminuria (urinary albumin excretion rate < 20μg/min) and serum creatinine ≤ 1.5mg/dL were randomly allocated

to placebo or active treatment with either verapamil (240 mg/day), trandolapril (2mg/day) or the combination of verapamil 180 mg/day and trandolapril 2mg/day. Since the effect of trandalopril and trandolapril/verapamil in delaying progression to microalbuminuria were similar, we combined both treatment arms in order to increase statistical power and compared against placebo. Patients with microalbuminuria at baseline were excluded from analysis. Urinary albumin excretion was assessed at randomization and every six months during the 3.6 years median follow-up.[4,11]

The DIRECT trial was a clinical trial program consisting of three clinical trials in 3326 type 1 diabetes patients with or without retinopathy and in 1905 type 2 diabetes patients with retinopathy. Patients had normo-albuminuria (urinary albumin excretion rate < 20μg/min) and serum creatinine ≤ 1.5mg/dL and ≤ 1.1mg/dL (for men and women respectively). In the DIRECT trial patients were randomly allocated to placebo or treatment with candensartan (16 mg/day increasing to 32 mg/day according to tolerability) and followed for a median time of 4.7 years.[12] Albuminuria was assessed at randomization and yearly during follow-up.[5] Patients with missing baseline albuminuria or microalbuminuria at baseline were excluded from analysis leaving 3095 type 1 and 1795 type 2 diabetes patients available for analysis.

The ALTITUDE trial was a randomized, double-blind, placebo-controlled trial enrolling 8561 patients with type 2 diabetes mellitus at high risk for cardiovascular and renal events. Eligible patients had either persistent macroalbuminuria (defined in this trial as urinary albumin/creatinine ratio (UACR) ≥200 mg/g), or an estimated glomerular filtration rate (eGFR) ≥30 to ≤60 mL/min/1.73m^2 combined with either persistent microalbuminuria (UACR ≥20 mg/g to ≤200) mg/g or a history of cardiovascular disease. Patients were randomly assigned to aliskiren 300 mg/day or matched placebo in addition to an optimal recommended dose of an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB). For the purpose of this study we excluded patients in the ALTITUDE trial who had UACR > 300 mg/g at baseline (n=4493) and those with missing baseline albuminuria measurements or no follow-up measurements (n=384) leaving 3654 patients available for analysis. Albuminuria was assessed at randomization, every 3 months during the first year and every 6 months thereafter. Randomized patients were followed for a median of 32.9 months for occurrence of cardiovascular and renal events.[6,16]

In the IRMA-2 trial, 590 patients with type 2 diabetes, micro-albuminuria (urinary albumin excretion rate > 20 to 200μg/min) and serum creatinine ≤ 1.5mg/dL and ≤ 1.1mg/dL (for men and women respectively) were enrolled. Patients were randomly allocated to either placebo or active treatment with irbesartan 150mg/day or 300mg/day. For the purpose of

analysis we combined the two irbesartan treatment arms to increase statistical power. Urinary albumin excretion was assessed at randomization and every six months during the 2 years follow-up.[3] We included 565 patients without macroalbuminuria at baseline.

Urine collections and albuminuria measurements

The frequency of urine collections (single, double, or triple) per study visit as well as albuminuria assessment (i.e. spot or timed collection) differed in the four trials. In BENEDICT and ALTITUDE three consecutive urine collections for albuminuria assessment were performed at each study visit. In DIRECT two consecutive urine collections were performed at each study visit starting the day prior to the study visit. In IRMA-2 only single urine collections were performed at each study visit. In BENEDICT and ALTITUDE urinary albumin and creatinine were measured in first morning void urine collections. In DIRECT and IRMA-2 timed overnight urine collections were performed and urinary albumin excretion was measured. In each trial urinary albumin and creatinine were measured in a central laboratory. In this study we defined microalbuminuria as a UACR of 30 mg/g or urinary albumin excretion of 20 µg/min. Macroalbuminuria was defined as a UACR of 300 mg/g or urinary albumin excretion of 200 µg/min.

Statistical analyses

Baseline characteristics are presented as mean and standard deviation for continuous variables and as counts and proportions for discrete variables. Non-parametric data are presented as median and interquartile range. The number of albuminuria class transitions (progression from either normo- to micro or micro- to macroalbuminuria) were recorded and incidence rate per 100 patient years was calculated. The effects of randomized treatment on all endpoint definitions were estimated from unadjusted Cox proportional hazard models, based on the intention to treat principle. For participants who experienced more than one event during follow-up, survival time to the first relevant endpoint was used in each analysis. Participants were censored at their date of death or, for those still alive, at the end of follow-up. The magnitude of the treatment effect is reflected by the hazard ratio and the precision of the treatment effect by its 95% confidence interval. We first determined whether increasing the number of consecutive urine collections at a single visit would lead to a more significant treatment effect. We subsequently determined the effect of the time-interval between visits, the addition of a confirmatory visit, and the addition of a percentage change in albuminuria from

baseline using the number of urine collections as defined in the first step. Unscheduled visits for albuminuria measurements were not included in the drug efficacy analyses.

The association between a transition in albuminuria class (progression to micro- or macroalbuminuria or regression to micro- or normoalbuminuria) during the first year of the trial and subsequent renal and cardiovascular outcomes was estimated using a multivariable Cox regression model. The Cox model was adjusted for age, gender, albuminuria, eGFR, systolic and diastolic blood pressure, HbA1c, and history of cardiovascular disease (yes/no).

All analyses were performed using SAS 9.3 for Windows (SAS Institute, Cary, NC). A two sided p-value < 0.05 was considered to indicate statistical significance.

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Conflicts of interests

TF Kropelin reports no conflicts of interests.

D de Zeeuw has consultancy agreements with the following companies: Abbvie, Astellas, Bristol-Meyers Squibb, Fresenius, Hemocue, Johnson & Johnson, Merck Darmstadt, Merck Sharpe & Dohme, Novartis, Reata Pharmaceuticals and Vitae. All honoraria are paid to his institution.

H-H Parving consults for AbbVie.

R Bilous reports honoraria from Roche, Novo Nordisk, Boehringer Ingelheim, Animas. He serves on data safety monitoring committees of clinical trials sponsored by Abbvie and Mitsubishi.

G Remuzzi has consultancy agreements with Dompé farmaceutici S.pA., AbbVie*, Alexion Pharmaceuticals*, Bayer Healthcare*, Reata Pharmaceuticals*, Novartis Pharma*, AstraZeneca*, Otsuka Pharmaceutical Europe*, Concert Pharmaceuticals*. *No personal remuneration is accepted, and compensations are paid to his institution for research and educational activities.

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Table 1: Baseline characteristics of the clinical trial populations

	BENEDICT (N=742)	DIRECT (T1D) (N=3093)	DIRECT (T2D) (N=1758)	ALTITUDE (N=3654)	IRMA-2 (N=565)
Gender, Male (n, %)	397 (53.5)	1771 (57.3)	858 (48.1)	2497 (68.3)	391 (69.2)
Age (years)	61.9 (8.0)	31.0 (8.4)	56.9 (7.7)	65.8 (9.4)	58.1 (8.6)
Diastolic BP (mmHg)	88.8 (7.7)	73.1 (6.9)	78.0 (7.0)	74.1 (10.0)	89.6 (9.3)
Systolic BP (mmHg)	152.4 (14.0)	116.9 (9.6)	132.7 (13.5)	138.2 (16.3)	152.7 (14.6)
Albuminuria*	5.2 [3.5 – 8.8] †	4.5 [3.5 – 7.0] †	5 [3.5 – 8.0] †	23.7 [4.11 – 73.6]#	58.0 [33.0 – 102.0]#
Normoalbuminuria (n,%)	742 (100)	3093 (100)	1758 (100)	1020 (27.9)	0 (0)
Microalbuminuria (n,%)	0 (0)	0 (0)	0 (0)	2634 (72.1)	565 (100)
HbA1c (%)	3.0 (1.4)	8.3 (1.6)	8.2 (1.6)	7.9 (1.7)	7.0 (1.7)
Tot. cholesterol (mg/dL)	211.8 (36.5)	184.8 (37.5)	204.9 (42.5)	170.5 (46.4)	223.2 (44.4)
eGFR (mL/min/1.73m2)	81.4 (15.3)	80.8 (13.7)	70.0 (14.2)	55.4 (20.6)	70.5 (12.4)

*Presented as median and IQR / #Urinary albumin:creatinine ratio mg/g / †Urinary albumin excretion rate µg/min

Table 2: Number of endpoints and event rates (events per 100 patient*years) for each clinical trial and endpoint definition

	Single visit			confirmation*
	1 measurement	Mean of 2 measurements	Mean of 3 measurements	1 measurement
Normo- to microalbuminuria				
BENEDICT	123 (5.9)	107 (5.1)	98 (4.1)	14 (0.7)
DIRECT (T1D)	441 (3.5)	344 (2.7)	Na	100 (1.0)
DIRECT (T2D)	431 (6.5)	380 (5.6)	Na	159 (2.9)
Micro- to macroalbuminuria				
IRMA-2†	114 (12.6)	Na	Na	26 (3.8)
Normo- to micro- and micro- to macroalbuminuria				
ALTITUDE	1861 (33.1)	1738 (29.4)	1717 (29.0)	1087 (21.0)
10% change in albuminuria in addition to a transition				
Normo- to microalbuminuria				
BENEDICT	123 (5.9)	107 (5.1)	98 (4.1)	14 (0.7)
DIRECT (T1D)	440 (3.5)	344 (2.7)	Na	100 (1.0)
DIRECT (T2D)	431 (6.5)	380 (5.6)	Na	159 (2.9)
Micro- to macroalbuminuria				
IRMA-2	114 (12.6)	Na	Na	26 (3.8)
Normo- to micro- and micro- to macroalbuminuria				
ALTITUDE	1858 (33.0)	1731 (29.3)	1713 (28.9)	1078 (20.7)
30% change in albuminuria in addition to a transition				
Normo- to microalbuminuria				
BENEDICT	120 (5.7)	103 (4.9)	95 (4.0)	13 (0.7)
DIRECT (T1D)	437 (3.4)	341 (2.6)	Na	99 (1.0)
DIRECT (T2D)	428 (6.4)	378 (5.6)	Na	154 (2.8)
Micro- to macroalbuminuria				
IRMA-2	113 (12.5)	Na	Na	25 (3.6)
Normo- to micro- and micro- to macroalbuminuria				
ALTITUDE	1826 (32.0)	1704 (28.6)	1684 (28.1)	1045 (19.8)
50% change in albuminuria in addition to a transition				
Normo- to microalbuminuria				
BENEDICT	112 (5.3)	100 (4.7)	88 (3.7)	13 (0.7)
DIRECT (T1D)	431 (3.4)	339 (2.6)	Na	99 (1.0)
DIRECT (T2D)	421 (6.3)	372 (5.5)	Na	146 (2.6)
Micro- to macroalbuminuria				
IRMA-2	109 (12.0)	Na	Na	22 (3.2)
Normo- to micro- and micro- to macroalbuminuria				
ALTITUDE	1754 (30.1)	1654 (27.2)	1636 (26.8)	989 (18.4)
100% change in albuminuria in addition to a transition				
Normo- to microalbuminuria				
BENEDICT	95(4.5)	80 (3.8)	73 (3.0)	8 (0.4)
DIRECT (T1D)	409 (3.2)	316 (2.4)	Na	93 (0.9)
DIRECT (T2D)	391 (5.8)	351 (5.1)	Na	131 (2.3)
Micro- to macroalbuminuria				
IRMA-2	95 (10.4)	Na	Na	20 (2.9
Normo- to micro- and micro- to macroalbuminuria				
ALTITUDE	1628 (26.5)	1504 (23.5)	1493 (23.3)	849 (15.3)

*Confirmation of transition endpoint at a subsequent study visit. The confirmation is based on a single urine sample obtained at the initial and confirmation visit.
†In the IRMA-2 trial only single urine collections were performed at each visit

Figure 1: Magnitude and precision of the drug effect estimate according the number of urine collections at a single study visit. Solid circles represents the estimate of the treatment effect and the horizontal line indicates the 95% confidence interval.

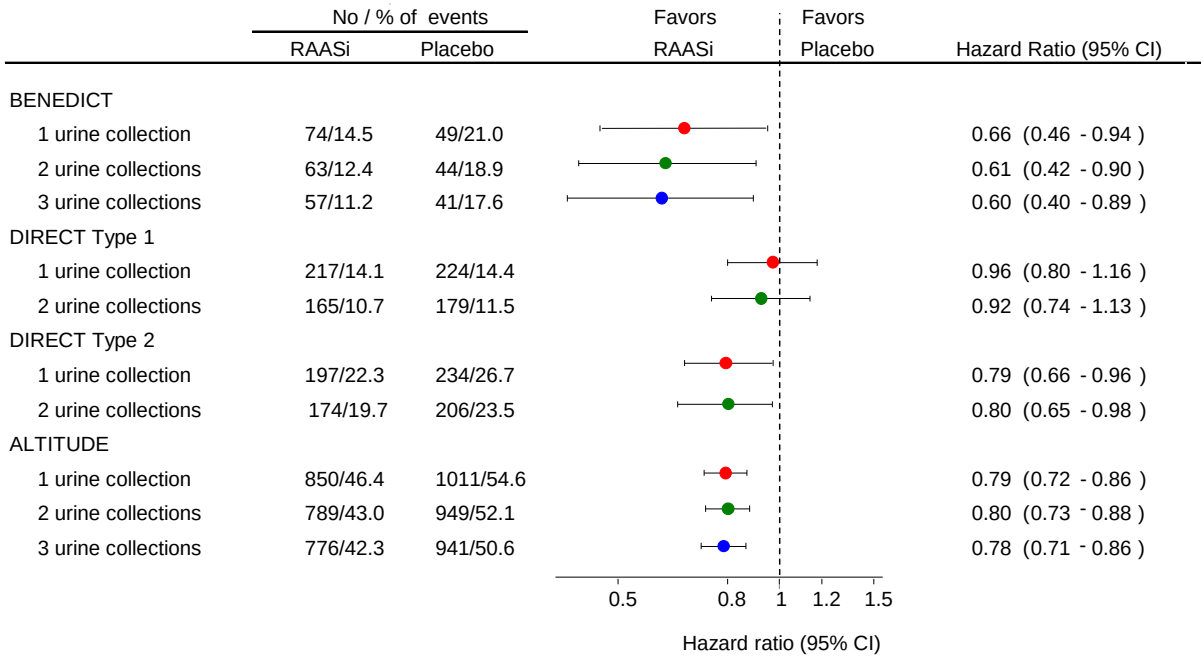


Figure 2: Magnitude and precision of the drug effect estimate with a single urine collection ~~and unconfirmed and~~with and without a confirmatory visit. Solid circles represents the estimate of the treatment effect and the horizontal line indicates the 95% confidence interval.

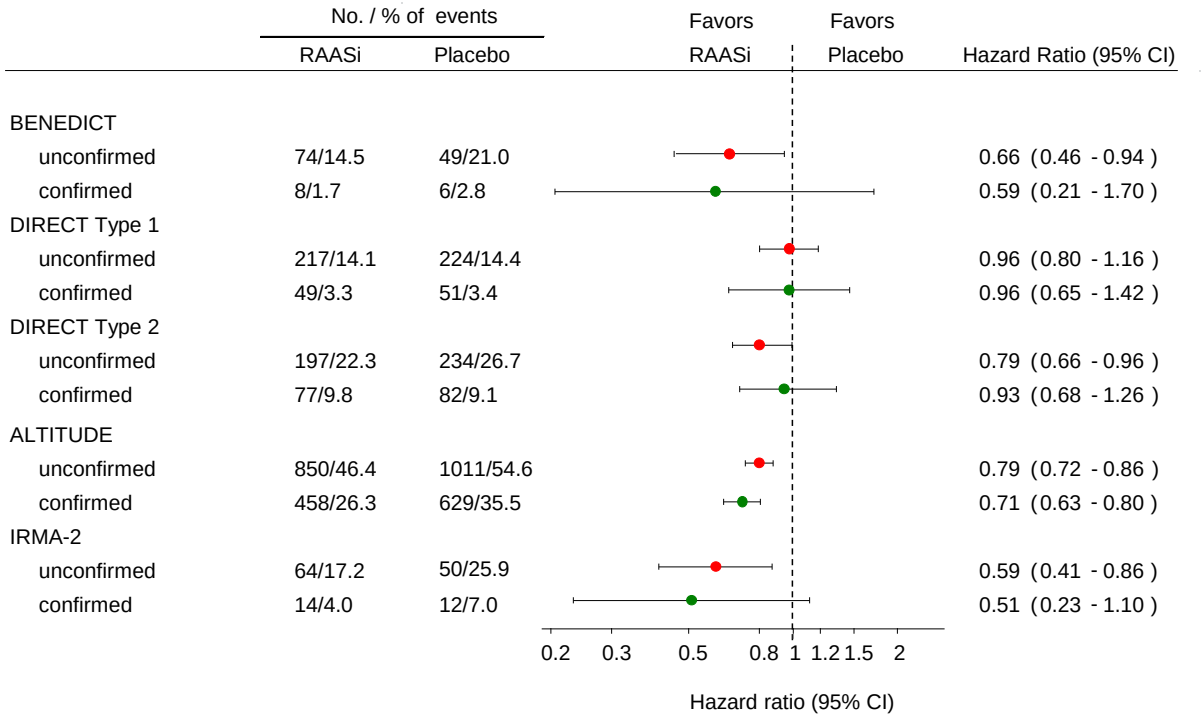
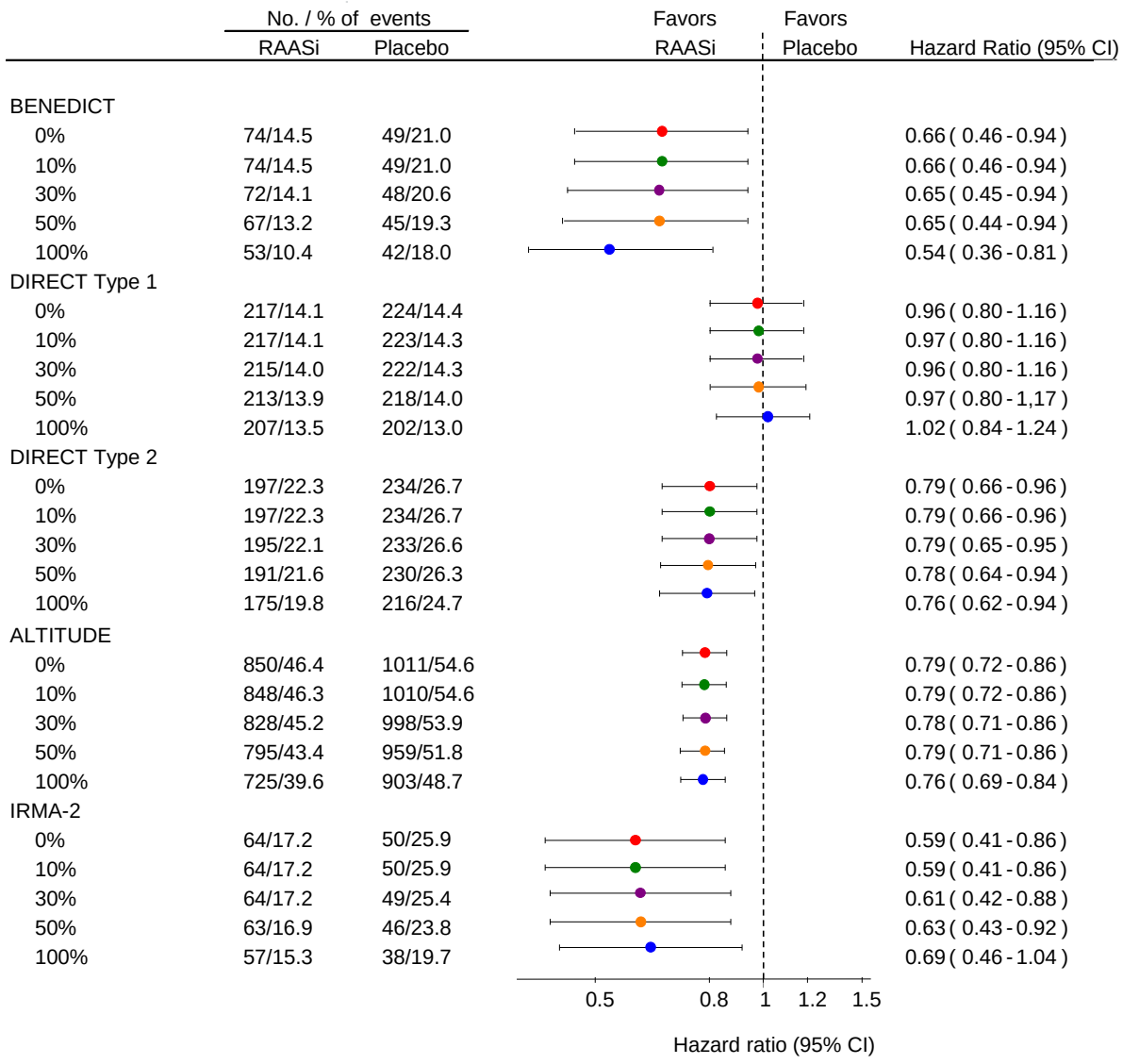


Figure 3: Magnitude and precision of the drug effect estimate with a single urine collection and various percentage ~~change in~~ albuminuria increases on top of the class transition without a confirmatory visit. Solid circles represents the estimate of the treatment effect and the horizontal line indicates the 95% confidence interval.



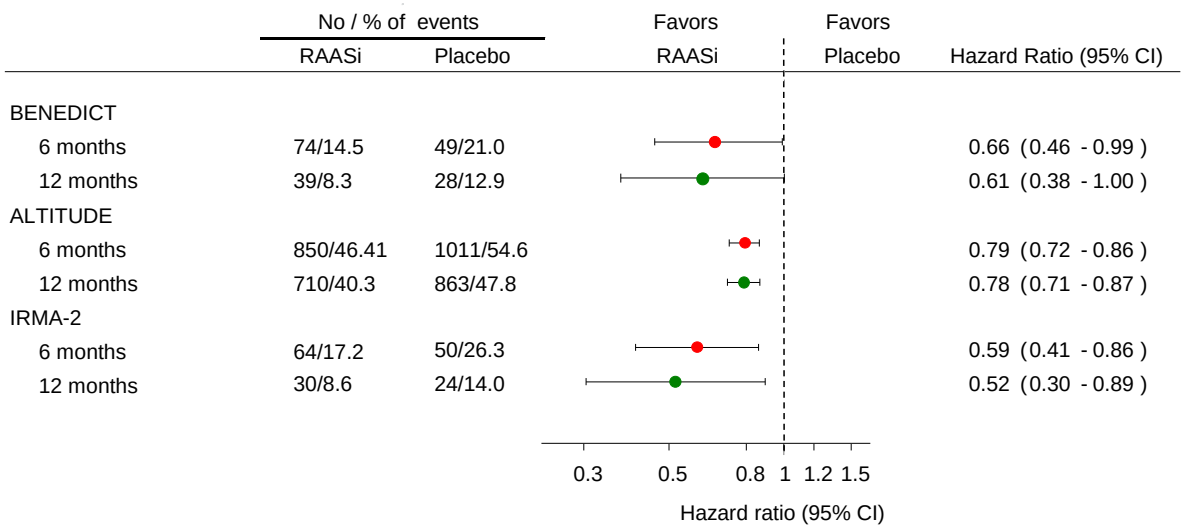
~~Supplement~~ **Table ~~1~~3:** Association between a transition in albuminuria class (normo- to microalbuminuria transition or micro- to macroalbuminuria transition) during the first year of the ALTITUDE trial with subsequent renal (doubling serum creatinine / end-stage renal disease / renal death) and cardiovascular (CV-death, resuscitated sudden death, myocardial infarction, stroke, unplanned hospitalization for heart failure) outcomes. The definitions of albuminuria class transitions are similar as those that were used to assess the drug effect.

	Renal outcome			***Cardiovascular outcome***		
	Hazard Ratio (95%CI)	*Chi^2*	*P*	*Hazard Ratio (95%CI)*	*Chi^2*	*P*
Single visit:						
Single urine sample	1.92 (1.09; 3.40)	5.02	0.025	1.45 (1.15; 1.83)	9.81	0.002
Two urine samples	1.80 (1.01; 3.20)	3.98	0.046	1.30 (1.02; 1.65)	4.46	0.035
Three urine samples	1.82 (1.02; 3.24)	4.12	0.042	1.35 (1.06; 1.72)	6.00	0.014
Addition of confirmation visit	2.47 (1.36; 4.49)	8.81	0.003	1.42 (1.09; 1.85)	6.84	0.009
Transition + 10% UACR increase	1.94 (1.10; 3.43)	5.19	0.023	1.44 (1.14; 1.82)	9.45	0.002
~~Transition + 20% UACR increase~~	~~1.97 (1.11; 3.48)~~	~~5.44~~	~~0.020~~	~~1.42 (1.12; 1.80)~~	~~8.65~~	~~0.003~~
Transition + 30% UACR increase	2.06 (1.17; 3.64)	6.20	0.013	1.39 (1.10; 1.76)	7.47	0.006
~~Transition + 40% UACR increase~~	~~2.13 (1.21; 3.76)~~	~~6.81~~	~~0.009~~	~~1.42 (1.12; 1.79)~~	~~8.43~~	~~0.004~~
Transition + 50% UACR increase	2.22 (1.26 – 3.91)	7.59	0.006	1.36 (1.07 – 1.72)	6.39	0.012
Transition + 100% UACR increase	1.98 (1.11 – 3.51)	5.42	0.020	1.30 (1.02 – 1.66)	4.39	0.036

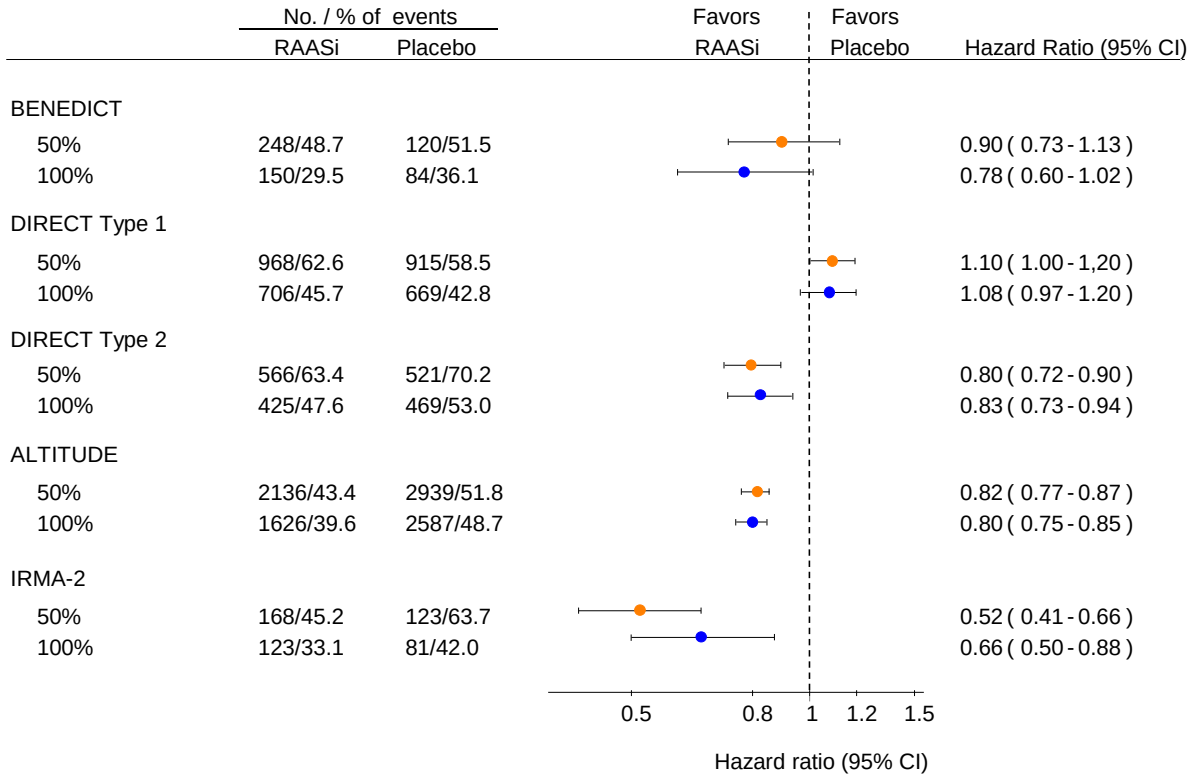
The presented hazard ratios are derived from a Cox proportional hazard models and adjusted for age, gender, albumin:creatinine ratio, eGFR, systolic/diastolic blood pressure, HbA1c, and cardiovascular disease history.

The composite renal endpoint was defined as end-stage renal disease, defined as the need for chronic dialysis or renal transplantation; renal death defined as the need for renal-replacement therapy with no dialysis or transplantation available or initiated; doubling of serum creatinine from baseline sustained for at least a month. The composite cardiovascular endpoint was defined as death from cardiovascular causes or the first occurrence of cardiac arrest with resuscitation; nonfatal myocardial infarction; nonfatal stroke; or unplanned hospitalization for heart failure. All clinical endpoints were adjudicated by a central endpoint committee utilizing standard definitions.

Supplement Figure 1: Magnitude and precision of the drug effect estimate with a single urine collection and different time-intervals between study visits. Solid circles represents the estimate of the treatment effect and the horizontal line indicates the 95% confidence interval.



Supplement Figure 2: Magnitude and precision of the drug effect estimate with a single urine collection and various percentage albuminuria increases *without* a class transition and without a confirmatory visit. Solid circles represents the estimate of the treatment effect and the horizontal line indicates the 95% confidence interval.



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